A STUDY OF SERUM COPPER AND ZINC
IN
NEW BORN BABIES

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C E R T I F I C A T E

This is to certify that the work entitled "A STUDY OF SERUM COPPER AND ZINC IN NEW BORN BABIES" which is being submitted as thesis for M.D.(Paediatrics) examination of Bundelkhand University, 1989 by DR. RAVI KANT, has been carried out in the department of Paediatrics, department of Biochemistry and department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi (U.P.).

He has put in the necessary stay in the department according to the University regulations.

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C E R T I F I C A T E

This is to certify that the work entitled "A STUDY OF SERUM COPPER AND ZINC IN NEW BORN BABIES" has been carried out by DR. RAVI KANT under my direct supervision and guidance in the department of Paediatrics with assistance of department of Biochemistry and department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi. He has fulfilled necessary requirements of the stay in the department for the submission of thesis. The techniques, observations and calculations incorporated in this thesis have been undertaken by the candidate himself and checked by me from time to time.

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A C K N O W L E D G E M E N T S

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C O N T E N T S

INTRODUCTION

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The trace elements are so called because they constitute less than 0.01% of the weight of the human body. However, despite their relative scarcity, their atoms are present in large numbers and each is believed to play an important role in human growth and development. The name "trace element" is, of course quite arbitrary and survives from the time when early investigators were experiencing great difficulties in measuring these substances, the introduction of more sophisticated techniques has resulted in an enormous increase in our understanding, of the role these elements play in metabolism.

About 30-40 percent of babies in India are low birth weight (LBW) as opposed to about 5 to 7 percent of new borns in the West. In India alone 7-10 million LBW infants are born annually. High incidence of LBW babies in our country is accounted for by a higher number of babies with intrauterine growth retardation (Small-for-dates) rather than the preterm babies. Preterm babies are anatomically and functionally immature and therefore their neonatal mortality is high.

Copper and zinc are essential metals required by the body in minute amounts. Both these elements have many biochemical roles, and function in the body as metalloenzymes, co-enzymes and as component atoms of physiologically important proteins and hormones. The

symptoms of deficiency of copper and zinc in adults are now well documented and three genetically determined disorders have been identified e.g. Menke's diseases, Wilson's disease and acrodermatitis enteropathica.

Little information is available on the possible role of trace elements in contributing to LBW. Nevertheless, such information is potentially of considerable importance because LBW increases excess mortality in infants.

Abnormal levels of some metals either in excess or a deficiency are known to affect fertility and produce embryogenic death, congenital malformation and neonatal diseases in experimental animals. Thus it was thought that the elements might also have an effect on the birth weight of the new born baby. Therefore, study of relationship of LBW and blood concentration of zinc and copper at the time of birth was undertaken. These metals were chosen primarily because of evidence and speculation in the medical literature suggesting possible relationships of these substances to neonatal morbidity and mortality in human or experimental animals.

The human fetus accumulates 70% of its copper and zinc body stores during last 12 weeks of gestation so, the infant born prematurely has small amounts of copper and zinc and may not develop his body stores in a way similar to the fetus.

Since, the human fetus accumulates some two thirds of its body complement of copper and zinc during the last trimester, the preterm infant acquires less than the normal amount and is dependent on its own ability to absorb these metals from the diet. Preterm infants on diets which contain inadequate levels of these metals exhibit symptoms of deficiency and may fail to thrive.

It is well accepted that a subtle relationship is maintained between mother and growing fetus. Any deficiency either of primary or secondary food factors including the trace metals in the mother is most likely to be reflected in the growing fetus which would adversely affected the latter and finally be manifested in new born. One of the important affected parameters of it is birth weight.

In the light of these observations the present venture is directed to measure the serum concentration of copper and zinc and to find out their relationship with gestational age, birth weight and also with sex of the child.

REVIEW OF LITERATURE

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The knowledge of trace elements remained almost stagnant till fiftees of this century. Even today it can be said to be in the adolescent stage. However, the value of trace elements in the aetiology and prophylaxis of various diseases has been realized today. Various workers have reported the involvement of trace elements in delayed wound healing, protein calorie malnutrition, growth failure, vision, anaemia, sexual infantilism and iatrogenic diseases due to prolonged therapy (Smith et al, 1973; Prasad et al., 1974; Underwood et al., 1977).

There are many elements in body tissue which play an important role in human growth and development. A full term infant comprising some 3.4×10^{26} atoms, contain 1.4×10^{22} trace element atoms. Zinc and copper are equally essential elements for the function of many enzymes. Only two decades ago scientists thought that zinc and copper played only some minor role in human system e.g. zinc with insulin and copper with cytochrome activities.

Zinc and copper deficiency in neonates have been reported and reports of zinc deficiency leading to dwarfism, delayed sexual maturation and intrauterine growth retardation are available. Plasma zinc and copper in Indian neonates have been reported but their relationship to sex, birth weight and gestational age has not been well studied.

HISTORICAL ASPECT OF ZINC

The geological presence of zinc is known from times immererial. It's use in medicine pottery and industry are also known from ancient time. Zinc is without doubt one of the essential trace elements in the living organism. It is present in most organs and fluids of plant, animal and organism.

Knowledge of the importance of zinc for the living organism began in 1869 when Raulin, a student of pasteur, found it to be necessary for the growth of black Mold Aspergillus niger. Subsequently, essentiality of zinc for higher plants (Mame, 1924) and animals was extablished. In the third decade of present century, the noted American scientist Sommer and Lipman (1926), demonstrated its presence in plants. Subsequently several workers reported the biological utility in several animals (Todd et al., 1934; Tucker and Salmon, 1955 and O'Dil, 1958 and Underwood, 1971).

The impetus for the study of the effect of the element on human biological process came about to some extent, through investigation of metal fume fever in the 1920's, when it was demonstrated that zinc oxide vapours were toxic to industrial workers (Sayers, 1938). In man the medicinal use of calamine (Zinc carbonate) was first recorded by papyrus Ebers of 1550 B.C. Eggleton (1939), suggested that zinc deficiency might contribute the clinical manifestation of human vitamin deficiency syndrome such as beriberi. Keilin and Mann (1940) discovered the

enzyme carbonic anhydrase from bovine erythrocyte, an enzyme that contains zinc. He offered the first explanation of the action of this element and a new field for investigations. Halsted et al (1972), identified the human zinc deficiency syndrome in malnourished adolescent boys in Iran and Egypt. It has been demonstrated that zinc is an integral constituent of, or cofactor for, more than seventy metalloenzymes (Riordan, 1976).

Clinical use of zinc began when an ancient egyptian, employed the oxide (or calamine) for treatment of burns and wounds. A scientific basis for its supposed efficacy was not available until 1953, when studies of experimental animals and wound healing in surgical patients demonstrated that zinc supplementation of the diet promoted healing (Pories et al, 1966). Prasad et al (1963), described the first syndrome of zinc deficiency of pediatric importance. Moyanhan and Barner (1973) showed more profound deficiency state when treatment with zinc induced a complete and rapid clinical remission in a patient with acrodermatitis enteropathica.

METABOLISM OF ZINC

Halsted et al (1974), found that serum concentration of zinc were higher than those of plasma. In blood 77-88 percent of zinc is present in R.B.C. while plasma zinc represents only 12-20%. He demonstrated the inhibition of formation of nucleic acid by the deficiency of zinc.

Evan et al (1977) showed in their study that breast milk contains low molecular weight zinc binding ligand that is absent from cow's milk. Hurley et al (1979) have identified this zinc binding ligand as zinc citrate and have suggested that zinc in cow's milk may be mainly casein bound and less available for absorption. Evans et al (1977) found that absorption of zinc is particularly poor from milk based on soy protein, which was also proved by Johnson et al in 1978. Phytate in the food interferes with the absorption of zinc. Although the zinc contents of cereals and vegetables is high, absorption is poor because of high phytate, fiber and cellulose content.

Walravens (1979), in his study showed that approximately 50% plasma zinc was freely exchangeable and loosely bound to albumin, 7% was bound to amino acids, and the remainder was bound to macroglobulins and other serum protein. Zinc has a role in the function of the important metalloenzymes including carbonic anhydrase, carboxypeptidase A and B, alkaline phosphatase, alcohol dehydrogenase, retinine reductase and lactic, glutamic and O-glyceraldehyde-3-phosphate dehydrogenase. It is also incorporated in a copper containing enzyme (Superoxidedismutase or the erythrocuprein).

Absorption of zinc, which probably involves active transport, occur mainly in the duodenum and proximal small intestine. Song et al (1978) has shown that prostaglandin E_2 augment the zinc absorption and its transport

across gut in the rat. On the other hand Indomethacin an prostaglandin inhibitor, was shown to inhibit zinc absorption. Zinc is mainly excreted by the intestine. Substantial amount of zinc is present in pancreatic secretion so absorption more than endogenous secretion is essential for positive zinc balance. Dietary zinc is taken up by the microvillus of mucosal cell. Inside the cell, zinc becomes associated with a number of ligands, probably low molecular weight ligand, which transport zinc to the serosal pole of the cell, from where it emerges to be bound to albumin. Metallothionein bind the intracellular zinc and control the zinc absorption by competing with the transport ligand for zinc. During excess of zinc in diet. synthesis of metallothianein is increased in liver and excess zinc is effectively bound. Significant amount of zinc is found in liver, bone,kidney, muscle and retina.

The National Academy of Sciences (1974) has recommended the following daily dietary allowances of zinc; infants, 3-5 mg; age 1 to 10 years, 10 mg; pregnant woman, 20 mg. Murphy et al (1975), in their study showed that the mazor dietary sources of zinc were foods of animal origin such as meat, fish, shell fish, poultry, eggs and dairy products.

Golden et al (1978) and subsequently Oleske et al (1979), reported that zinc also plays some role in the immune system of body. The effect of zinc on host defence response is as follows : (1) contributes to plasma

membrane integrity and the functioning of binding sites. (2) It inhibits phagocytic function in high concentrations. (3) Low body stores of zinc are associated with dysfunction of T. cell (Beisel, 1977).

Solomons (1979), reported that plasma level of zinc fall in patients with infection, inflammation or stress due to leucocyte endogenous mediator. Indirect test for diagnosis of zinc deficiency are a change in alkaline phosphatase, red cell carbonic anhydrase activity, defective taste threshold, impaired macrophage hemotaxis and decreased salivary and skin zinc.

Hambridge (1979), found that zinc levels in human colostrum range between 10 and 20 mg/l, while the concentration in milk was noted to remain at 3 mg/l during the first month and then decline with prolonged lactation. Concentration of zinc is much higher in colostrum than in mature breast milk. Absorption of zinc from intestine is enhanced by zinc binding ligands which are present in human milk and which protects the premature infant and breast fed baby against zinc deficiency.

Rajalakshmi et al (1980), in their extensive work studied the copper and zinc content of breast milk of Indian women and observed that copper levels fell from 0.46 ug/ml in colostrum to 0.17 ug/ml at 7 to 12 months of lactation; zinc levels fell from 5.32 to 1.12 ug/ml by 7 month. Concentration of serum was not correlated with those of milk which suggest that the transfer of these elements from plasma to milk is not a passive process but

there were some active mechanisms involved. The growth and clinical condition of the infants of study group were satisfactory, suggesting that the copper and zinc requirement of Indian infants were adequate.

ZINC DEFICIENCY

Prasad et al., (1963) were perhaps the first worker to have described the zinc deficiency with clinical significance in boys from middle East with syndrome of poor growth, hypogonadism, anaemia and geophagia. Treatment with zinc was followed by dramatic increases in growth and sexual maturation.

Agett et al., (1979) in their extensive work on zinc states in health and disease reported that zinc deficiency can occur due to decreased intake, consumption of vegetarian diets, babies on synthetic or soyabean formula, malabsorption, increased body losses and parenteral hyperalimentation. The early manifestations of zinc deficiency are anorexia, growth retardation and impaired taste and olfactory sensation. Other features are pica, hypogonadism, behavioural abnormality, neurological features (intentional tremor, nystagmus, dysarthria, jitteriness), night blindness, skin lesions, delayed wound healing, diarrhoea and impaired cell mediated immunity.

In subsequent study, later on, Agett et al (1980), described symptomatic zinc deficiency in a breast fed preterm who developed severe symmetrical facial

dermatitis at 2 month of age. Diagnosis of acrodermatitis enteropathica was excluded. Oral zinc supplements induced a complete remission. Author inferred, that low intake, perhaps, associated with impaired absorption of zinc caused the zinc deficiency in their patient.

Gordon et al. (1981), described zinc deficiency states with cystic fibrosis, Fetal alcohol syndrome, Acne, sickle cell anaemia, nephrotic syndrome and schizophrenia.

Kumar et al. (1984), reported edema, hypoproteinemia and zinc deficiency in three premature infants as an unusual presentation between 5 and 8 weeks of age. The infants were fed mothers milk, supplementation with a proprietary formula was done after first 2-3 weeks of life. Treatment with oral zinc led to rapid resolution of edema, with increase in serum protein. Author suggested, that zinc plays an important role in the synthesis of nucleic acid and protein. It was postulated, that dietary zinc deficiency in the phase of rapid postnatal growth precipitated oedema and hypoproteinemia in these infants.

ZINC TOXICITY

Aggett (1979), described toxic effects of oral ingestion of zinc as nausea, vomiting, lethargy, dizziness, diarrhoea, and bleeding gastric erosion. Acute renal failure and death may occur after intravenous over dosage. Contact dermatitis to zinc salts and zinc allergy in diabetes on zinc insulin preparation have also been described.

COPPER METABOLISM

Copper is a component of hemocyanin, the respiratory pigment of snails, which was known for more than 100 years. Hart et al. (1928), were the first workers to have noticed that copper has a biochemical function in mammals. Copper was found to be essential, alongwith iron for normal erythropoeisis, since that time copper has been shown to be a component of several enzymes ofkey importance in metabolism. It's nutritional value was also stressed by the worker.

The daily requirement of copper vary between 0.08 mg/kg of body weight for infants and 0.03 mg/kg for adults (WHO technical series, trace elements in human nutrition, 1973, p. 532). Like zinc, requirement and processing of foods lead to a reduction in copper cotent. Meat, fish and green leafy vegetables are rich in copper content.

Solomons (1977), reported that copper by forming complex with amino acid is absorbed by active process and some of it is associated with metallothionein, in the mucosal cell. The copper is transported in the blood to the liver, where ceruloplasmin synthesis takes place. The principal route of excretion of copper from the body is bile where it is found complexed to proteins and low molecular weight ligands. The protein bound copper in bile is apparently poorly reabsorbed, while the remainder enter into an entero hepatic circulation. Liver is a major storage site for copper whereas in plasma more than

90% is found as ceruloplasmin. The remaining part is loosely bound to albumin and amino acid.

Bonta et al. (1977), reported that concentration of enzyme super-oxide dismutase is high in RBC of the foetus and falls towards term. Forty percent of RBC copper is in labile pool which is in equilibrium with plasma copper. In plasma about 96% of the copper is present as ceruloplasmin. Majority of copper not bound to ceruloplasmin is bound to serum albumin. The albumin bound copper is labile. Plasma copper levels are higher in women that in men. During pregnancy, copper and ceruloplasmin levels rises. Hussain et al. (1982) reported, that premature babies had definitely low values of ceruloplasmin as compared to full term babies. They found positive correlation with birth weight. In case of neonatal infection, the worker observed, significantly high values at first and second week of age, which returned to normal at one month. Copper is a component of a number of copper metalloenzymes. He further suggested that function of copper is related to its ability to engage in oxidation reduction reaction. Highest level of copper and ceruloplasmin are seen at term which fall to normal by about 6 weeks after delivery.

Nassi et al. (1979), noticed that the concentration of copper, unlike that of zinc, seems to be no higher in colostrum than in mature milk.

DEFICIENCY OF COPPER

Hart et al. (1928) and subsequently Elvehjem (1935), showed that copper might be affective in treating the anaemic infants. This work was followed by number of reports of hypocupremia in anaemic infants, some of them responded to therapy with copper (Sturgeon et al, 1956; Schubert et al., 1959).

Graham and Cordano (1976), reported that malnourished infants rehabilitated on cow's milk diet low in copper, developed copper deficiency. Their serum copper and ceruloplasmin level fell significantly. Infants developed neutropenia and anaemia, the latter being typically resistant to iron therapy. Later on they developed bone changes and occasional fractures. They reported that principal features of copper deficiency syndrome are psychomotor retardation, hypotonia, pallor and hypopigmentation, prominent scalp veins in palpable periosteal depressions, hepatosplenomegaly, roentgenographic changes with osteoporosis, blurring and cupping of metaphysis, fractures, bone marrow showing vacuolated erythroid and myeloid cells with iron deposition in vacuoles and neutropenia.

Sann et al. (1978), reported a case of copper deficiency associated with hypocalcemia, radiological fractures of rickets and hyperparathyroidism in small for date infant, whose gestational age was 39 weeks and weight 1,240 gm at birth. Serum copper was low along with features of rickets. Vitamin D_2 was given to infant which induced

an immediate normalization of serum calcium and normal X-rays. Serum copper and ceruloplasmin levels increased slowly. These results suggested a role of copper deficiency in the occurrence of this trasient vitamin D resistant rickets.

Yuen et al. (1979), reported copper deficiency in a infant of very low birth weight. It was characterized by extensive bone changes, severe neutropenia and hypocupremia.

Sutton et al. (1985), more recently reported a infant of very low birth weight (less than 1500 gm) who developed signs of copper deficiency between 8 and 10 weeks. It was characterized by osteoporosis, oedema, anaemia, neutropenia and late apnoea which improved when the oral copper intake. was increased.

SERUM ZINC AND COPPER IN NEWBORN BABIES

Many workers in the recent past have reported that the level of both zinc and copper in cord blood are related directly to the gestational age and birth weight of new born baby. Low birth weight and prematurity is the major cause of perinatal deaths. Many workers have suggested that unblanaced mineral nutrition including the trace elements could be the cause of low birth weight babies. Plasma zinc and copper in Indian neonates & their relationship to sex, weight and gestational age has not been well studied.

In view of this the present study was undertaken to ascertain the relationship of zinc and copper with gestational age, birth weight and sex of the baby.

Prasad et al (1961), were perhaps the first workers to have suspected the zinc deficiency in paediatric group and later on established it in the year 1963. They reported that the syndrome of zinc deficiency was characterized by poor growth, hypogonadism, anaemia and geophagia.

Neri et al (1969), were perhaps the first workers to have extensively studied copper along with copper oxidase content of maternal and infant umbilical artery and venous blood serum at delivery. One hundred parturient women and their new borns were included in the study group. Average maternal serum copper level were found to be 270 ug% at delivery. The serum copper content of the new born was 68 ug% and 65 ug% in venous and arterial blood respectively which were almost similar.

Worker observed no correlation between age, parity and weight of the baby to serum copper and copper oxidase level. The average value for serum copper and copper oxidase in the maternal blood was found to be about 4 times higher than that observed in the new born babies.

Author opined that the hypocupremia of the new born serum was probably due to two causes (1) The binding of copper to copper oxidase and (2) The

inability to synthesise the cuproprotein which is due to immaturity of the fetal liver. They further stated that hypercupremia of pregnancy is due to mobilisation of copper from maternal tissue. Effect of hormone has also been attributed as a cause of elevation of maternal serum copper level.

Worker observed higher concentration of copper in placenta (227 ug%). It would seem that the placenta, like the liver is a store house for copper, a fact, which may explain its higher concentration there in.

Another important finding observed by them were significant elevation of serum copper and copper oxidase levels in maternal and new born serum levels of six parturients who had pre-eclampsia at the time of delivery. They opined that hypercupremia of toxaemia is a compensatory mechanism in which copper ions improve oxygen utilization in the tissues, increases glycolysis in muscular extract and inhibit heavy metal poisoning of the enzyme. Since the liver is frequently affected in toxaemia of pregnancy, the cause of hypercupremia is attributed to subclinical hepatic damage.

Henkin et al (1971), in their study on maternal fetal metabolism of copper and zinc at term investigated 15 normal mother and their 15 normal babies. At delivery venous blood was obtained from mother and from the umbilical cord of their babies. Estimation of copper and zinc was done by atomic absorption spectrophotometry. Estimation of these elements were also done in the amniotic fluid.

The author observed, that the serum concentration of copper in mothers at term were mean (± 1 S.E.M.) 221±14 ug/100 ml (Normal nonpregnant mean values being ± 1 S.E.M., 107±3 ug/dl), which were approximately 2½ times the normal non pregnant adult female values. Mean concentration of free copper in maternal pregnant serum (8±1 ug/dl) was not significantly different from non pregnant level (12±2 ug/dl). Total serum concentration of zinc in mother at term were lower (Mean±1 SEM 48±3 ug/dl) than non pregnant level of zinc (90±3 ug/dl), while concentration of free zinc in the pregnant women at term were about 2½ times the non pregnant value.

Mean concentration of total copper and zinc in fetal serum were 29±3 ug/dl and 83±3 ug/dl respectively with free copper and zinc 16 and 32 percent of their respective total concentration. Mean concentration of total copper in fetal serum were much lower than the mean concentration of serum copper in mothers and non pregnant women, while zinc concentration in babies at birth were almost equal to adult value and approximately twice that of its concentration in mothers at term.

Author observed that mean concentration of total copper and zinc in amniotic fluid were 6 and 32 ug per 100 mb respectively with free copper and zinc 83 and 92% of their respective total concentration.

Workers found no significant differences in copper or zinc in serum of mother or fetus relative to

fetal sex. They opined that due to increase in ceruloplasmin bound copper as a result of elevated levels of maternal estrogen, there were increase in total copper in maternal serum. While lowered levels of total zinc in maternal serum at term were due to diminished quantities of zinc binding proteins or alteration in binding affinities.

In fetus, all bound copper is carried by ceruloplasmin. Since mean total serum zinc concentration of both bound and free form are not significantly different from adult, those factors, which alter zinc metabolism in mother at term were apparently not active in fetus. Author opined that copper and zinc both move across placenta possively . Presumably, since only free metals are available for transfer. There are maternal fetal gradients for copper and zinc of 13 and 5 ug per 100 ml respectively. The metal concentration in amniotic fluid are compatible with their passive transfer into this fluid.

Schenker et al (1972),in their extensive work on maternal and serum copper levels at delivery included 160 parturients having uncomplicated antenatal course of pregnancy, who delivered normally at term. Serum copper was also estimated in maternal and cord blood in complicated pregnancies.. These included : 48 premature babies, 36 new borns weighing 1,500-2,499 gm and 12 new born weighing less than 1,500 gm and 26 cases of clinically established postmaturity. Mothers having pre-eclampsia,

Rheumatic heart disease, diabetes treated with insulin and delivered by caesarian section and cases of postmaturity were also included in the study group. Serum copper was also studied in babies suffering from Rh incompatibility congenital malformation, an-encephaly heart and great malformation and diaphragmatic hernia.

Maternal blood was obtained from antecubital vein at delivery and cord blood was withdrawn immediately after birth. Estimation of copper was done by atomic absorption spectrophotometry.

Author observed that average value of serum copper in normal pregnancy was 275 ug%. SD±10.1. There was no difference in serum copper of new born arterial (56 ug%) and venous (59 ug%) cord blood at term delivery. In 48 cases of premature deliveries (32-36 weeks gestation age) mean maternal serum copper level was 250 ug%., SD±35. Which was lower than normal pregnancy. While average serum copper level of cord blood shows no significant difference (56 ug%, SD±11.3).

In complicated pregnancies maternal blood showed higher serum copper level in pre-eclamptic mothers (340 ug%, SD±38.3), in diabetic mothers (285 ug% 36 weeks gestation), in mothers having RHD (290 ug%) t than the serum copper values in normal pregnancies at the same stage of gestation. While no significant elevation was noticed in the offspring. Cord blood level in toxaemia was 59 ug% SD±14.1 and in RHD was 57 ug% SD ± 9.8.

In contrast, average serum copper level in case of postmaturity was lower in mother (240 ug%, SD±40.3), while 10% increase was found in serum copper level of cord blood (65 ug%, SD±15.6). New born suffering from Rh incompatibility also showed higher level of serum copper in cord blood (73 ug%±18.3 SD).

Author opined that hypercupremia of the new born is due to reduced ability of immature fetal liver to synthesize ceruloplasmin to release the synthesized protein into the blood stream. The difference in serum concentration in male and female at birth was insignificant. Higher content of serum copper in eclamptic mother may be attributed to subclinical hepatic damage. Slight hypercupremia (13%) noticed in serum cord blood of new born suffering from Rh incompatibility may be due to haemolytic process. The worker inferred that serum copper determination in maternal serum and in cord blood does not help in the detection of congenital malformations.

Kurz et al, (1973), in their study tried to establish normal values of serum zinc in new born and tried to find out the variation, if any, according to sex and race. Cord blood was collected at the time of delivery. 1 ul of serum was required for determination of zinc level by atomic absorption spectrophotometry. Normal adult control serum was used as a check on day to day variation in the method. No significant variation was found. For statistical analysis the study group was

divided into four smaller groups based on race and sex (1) Caucasian male (2) Caucasian female (3) Negro male (4) Negro female).

Author observed that the serum zinc value (Mean±SD) for whole of the group was 91.8±14.4 ug/100 ml (n=115), whereas for adult controls it was 94.6±11.0 ug/100 ml. Statistically no significant difference occurred between caucasian males and Negro males (p=0.30) caucasian females and Negro females (p=0.10) between the two extreme values (Caucasian males and Negro females) (p=0.10) or between the newborns and adult (p=0.40).

Prasad et al (1974), studied the maternal fetal interrelationship of zinc by estimating the level of zinc in maternal serum at term, in cord blood and in liquor amnii drawn during the process of birth. Worker included forty four pregnant women and ten non pregnant women in their study. Venous blood from forty four mothers at term and the cord blood of their babies were collected. The liquor amnii was collected by puncturing the membrane in thirty six subjects at term just before delivery. For control venous blood of ten nonpregnant women were collected.

Author observed that mean plasma zinc level in pregnant and nonpregnant women were 70.0±18.7 ug/100 ml and 118.3±29.4 ug/100 ml respectively. Level of zinc in pregnant women were significantly lower than that of nonpregnant women ($P < 0.001$). Mean plasma zinc level in

the cord blood of 64 normal healthy neonates was 100.8 ±34.5 ug/100 ml and in small for date neonates (Birth weight 1,900 gm - 2,900 gm) was 97.5±26.5 ug/100 ml. Statistically, the difference between the two was not significant. Plasma zinc level was also compared in the mothers of healthy neonates and those of small for date neonates. Mean plasma zinc level in 24 mothers of healthy neonates and 20 mothers of small for date neonates was 71.6±19.2 ug/100 ml and 68.0±18.6 ug/100 ml respectively. Difference was statistically not significant.

Zinc concentration was also estimated in liquor amnii which was collected during the process of birth by rupturing the membrane. Thirty six samples of liquor amnii were collected, twenty were from the mothers, who gave birth to normal healthy neonate, while sixteen were from those, who gave birth to small for date neonates. The mean zinc level was 25.2±13.8 ug/100 ml in the mothers of normal healthy neonates and 32.3±23.9 ug/ 100 ml in the mothers of small for date neonate. Statistically, the difference between the two groups was not significant.

Worker also compared the total zinc level in cord blood, maternal blood and in amniotic fluid with respect to the sex of the baby. In twenty two female new born, mean plasma zinc level in cord blood was 73.2±24.5 ug/100 ml, while in twenty two male neonates

it was 83.7±83.7 ug/100 ml. It was statistically not significant. Blood level of zinc in those mothers (43.0±23.2 ug/100 ml) who gave birth to female child was significantly lower ($P < 0.01$) as compared to those who who gave birth to male child (62.3±15.6 ug/100 ml). Zinc level in amniotic fluids of mothers of female child was higher (27.3±8.5 ug/100 ml) than those of male child (16.3±2.4 ug/100 ml).

Author opined that decreased zinc binding capa-city in pregnant women was the cause of lower level of plasma zinc. Mean total plasma zinc of the mother at term was 71.6±19 and the mean concentration of total zinc in their amniotic fluid was 25.2±13.8 ug/100 ml. Worker suggested that it is the free form of plasma zinc which is transferred from mother to fetus, and small quantity of zinc in amniotic fluid was probably the fetal urine which was excreted in the amniotic fluid. Similar plasma concentration of zinc in full term neonates and small for date neonates were due to similar zinc binding capacity in both the cases.

Author inferred that female fetus needs more zinc for growth than male fetus, by the fact, that plasma zinc level were lower in pregnant women who gave birth to female child than in those who gave birth to male child. It was supported by the fact that zinc concentra-tion was higher in amniotic fluid of mother of female child than in those who gave birth to male child.

Bogden et al (1978), carried out copper, lead, iron, magnesium and calcium analysis in maternal and cord blood in low birth weight group in order to delineat possible relationship between low birth weight and above said blood metal concentration and also investigated relationship between maternal and cord blood. For this purpose they selected 25 women giving birth to infant weighing between 1,500 gm to 2,500 gm and 50 women giving birth to infant weighing more than 2,500 gm. The cases and controls were matched for age (±4 years). Analysis of variance testing of the effect of infant sex and maternal parity on each of the five metal concentrations were carried out for both maternal and cord blood. There was no significant ($P > 0.10$) relationship between infant sex or maternal parity and any of the maternal or cord blood concentrations.

Author observed that mean plasma copper concentration in maternal blood of 25 low birth weight group cases and 50 normal birth weight group controls, were 174±26 ug/100 ml and 168±40 ug/100 ml respectively. In cord blood its concentration was 84±16 ug/100 ml in cases and 80±19 ug/100 ml in controls. So, no significant differences between the low birth weight and control group were found for copper in either maternal or cord blood. Worker observed that significant differences ($P < 0.001$) were found for calcium, with the low birth weight group having both lower cord and lower maternal calcium concentration (maternal 5.40±0.47 ug/100 ml in cases and 6.13±0.87 ug/100 ml in controls, cord

5.21±0.64 mg/100 ml in cases and 6.01±1.20 mg/100 ml in controls).

Spearman correlation coefficient between metal pairs were calculated. The significant correlation were maternal copper with cord copper (r = 0.26), maternal magnesium with cord magnesium (r=0.26), maternal calcium with cord calcium (r=0.38) maternal lead with cord lead (r=0.55) and cord iron with cord magnesium (=0.37). Out of these five significant correlation, four are correlation between the concentration of metal in maternal blood and the concentration of same metal in cord blood, illustrating strong dependence of the fetal concentration on the maternal concentration.

Author concluded in their study that there is no significant difference in maternal whole blood copper of low birth weight group and normal birth weight group. While in study of Schenker et al (1972) maternal serum copper level were lower in cases of prematurity. Author interpreted that this difference may be due to the use of whole blood in their study instead of serum. They inferred that fetus is dependent on maternal copper as they found significant correlation between maternal and cord blood copper.

In subsequent study Bogden et al (1978) investigated the plasma concentration of calcium, chromium, copper, iron, magnesium and zinc in maternal and cord blood and also studied their relationship to low birth weight. Maternal and cord blood were collected at the

time of delivery. Patients sample were divided into two groups, the low birth weight group (group A, 22 cases) who had birth weight 1500 gm - 2,500 gm, the normal birth weight group (B) comprised of those with birth weight greaper than 2,500 gm. Subgroup of the 50 controls (B2) comprised of the 22 normal birth weight deliveries most closely matched to each of the 22 low birth weight deliveries for infant sex and maternal age, race, smoking habits and parity.

Estimation of trace elements was done by atomic absorption spectrophotometry.

Author observed that mean±SE plasma concentration of copper in group A, B1 and B2 in maternal blood was 229±14 ug/100 ml, 199± 12 ug/100 ml and 206±10 ug/100 ml respectively. While cord blood copper level was mean ±SE 51±6 ug/100 ml in group A, 51±4 ug/100 ml in B, and 52±5 ug/100 ml in B2. On statistical analysis level of plasma copper in both maternal and cord blood was insignificant in group A, B1 and B2. Plasma copper level in maternal blood at term was about 4 times more than that in cord blood. Therefore author inferred that the magnitude of the rise in plasma copper during pregnancy might be related to the ability of the pregnant women to meet the demands of the growing fetus for copper.

Mean plasma concentration of zinc in maternal blood in group A, B1 and B2 were 64±2 ug/100 ml, 62± ug/100 ml and 62±2 ug/100 ml respectively. In cord blood

zinc level was 105±7 ug/100 ml in group A; 103±4 ug/100 ml in group B1 and 100±3 ug/100 ml in group B2. Statistically plasma zinc level in maternal and cord blood in all the three group i.e. A, B1 and B2 were not significantly different. In cord blood plasma zinc level were higher than that in maternal blood which was in agreement with the result of other workers.

Author inferred that many factors acting additively or synergistically can produce low birth weight and the results of this study suggest that nutrient metals may be one of these factors.

Atinmo et al (1980), investigated zinc and copper status of maternal and cord blood and also their relationship with birth weight. Worker included twenty women who gave birth to infants weighing 1,500 - 2,500gm (cases) and thirty women who delivered infant weighing more than 2,500 gm (controls).

Analysis of zinc and copper in plasma samples were done by atomic absorption spectrophotometry.

Author observed that zinc level in the cord blood were higher than in the maternal blood and that the mean plasma zinc in maternal and cord blood were significantly lower in the low birth weight as compared to normal control group of average birth weight. In their study, the zinc levels were 73.15±23.5 ug/100 ml in normal(Control) group and 66.31±14.46 ug/100 ml in low birth weight group. In cord blood, the levels were

89.52±13.01 ug/100 ml in normal (control) group and 83.37±15.00 ug/100 ml in low birth weight group. In maternal blood copper levels were 221.86±22.77 ug/100 ml in cases and 203.59±36.41 ug/100 ml in controls, while in cord blood copper levels were 60.29±18.34 ug/100 ml in cases and 56.48±17.59 ug/100 ml in controls. Maternal and cord blood copper levels were significantly higher ($P < 0.05$) in the cases than in the controls.

Maternal/cord blood concentration ratios for the cases and controls for copper and zinc was also studied and only the maternal/cord blood ratio for copper was significantly different ($P < 0.05$), being 4.59 for the cases and 4.04 for the controls.

Effect of parity on the concentration of each trace element was also studied but no significant relationship was found.

Author interpreted that higher levels of zinc in cord blood in their study suggested an increasing transfer of zinc to the developing fetus. For the explanation of low level of copper in cord blood, it was believed that copper can not diffuse across the placenta but accumulates to the layers of the placenta from where it is transfered to fetus by an active process according to need. Author think of possible role of trace elements in the occurrence of low birth weight among nigerian babies as they found significant correlation between birth weight and trace metal concentration.

Hillman (1981), estimated the serial serum copper concentration in premature and small for gestational age (SGA) infants during the first 3 month of life. The aim of study was to collect the normative data of serum copper concentration in premature and SGA infants within the first three month of life and to obtain the knowledge of the copper balance during this period.

Author studied 57 premature infants for at least 3 weeks. Infants were classified into preterm, term AGA and term SGA. In preterm infants of 25-28 weeks of gestational age mean serum copper level in cord blood was 22.1±13.2 ug/dl, in 29-30 weeks age 23.3±17.2; 31-32 weeks of age 25.1±18.3 ug/dl; 33-34 weeks age 33.5±20 ug/dl. Total mean serum copper concentration in cord blood of preterm infants was 26±16.8 ug/dl. In mothers of preterm infants mean serum copper concentration were higher (139±25.4 ug/dl) than that of newborn. In twenty two term SGA infants mean cord blood copper concentration were 50±21 ug/dl and in term AGA infants mean level of copper were 32±21 ug/dl. Serum copper level in mothers of term AGA and term SGA infants were similar to the mothers of preterm infants (140±30 ug/dl in term SGA; 150±25 in term AGA).

Post natal study of serum copper concentration in all groups of infants were studied and all infants were fed a formula containing 0.4 mg/l copper, which would yield a copper intake of 70-90 ug/kg at 120 to

150 calories/kg. Serum copper was measured by atomic absorption spectrophotometry.

Both premature and term infants had a significant increase in serum copper concentrations between birth and one week ($P < 0.01$). Although the mean±SD slowly rise in premature infants, no further significant increase in serum copper concentration occurred until 12 weeks, when again a significant ($P < 0.01$) increase occurred. The SGA infants always had a higher serum copper concentration and most increased the value by 6 weeks of post natal age. Term infants reached normal adult serum copper levels by one month of age. In very premature infants, these increases were delayed for three to four months.

Author opined that delayed increase in serum copper level in premature infants were possibly due to the immaturity of gastrointestinal tract affecting the absorption of copper. Second possibility that he explained was the immaturity of liver for the production of ceruloplasmin. Level of ceruloplasmin were found to be lower in preterm cord blood.

At 3 month of age it was expected to have an increase in serum copper and ceruloplasmin concentration, so author suggested that very premature infants in whom the serum copper concentration was found to be very low, may be copper deficient, especially if clinically compatible and may benefit from additional copper supplies.

Goel and Misra (1982), investigated plasma zinc in neonates and their mothers. They included sixty five neonates and classified them into full term appropriate for gestational age (AGA twenty five neonates) Full term small for gestational age (twenty neonates) and premature appropriate for gestational age (twenty neonates). The levels of zinc were also estimated in the mothers of the three groups at the time of delivery and were compared with the cord blood levels and those in non pregnant mothers. Estimation of plasma zinc was done by atomic absorption spectrophotometry.

Author observed that mean value of plasma zinc obtained for the premature AGA infants (90.2±5.25 ug/dl) were significantly lower than the mean plasma zinc level in full term AGA INFANTS (111.1±9.35 ug/dl) and full term SGA (106.57±7.39 ug/dl). Plasma zinc level in mothers of full term AGA infants, full term SGA and preterm AGA infants were 76.3±5.64 ug/dl, 72.6±6.08 ug/dl and 84.20±4.33 ug/dl respectively, which indicated that plasma zinc level in pregnant mothers were significantly lower as compared to neonatal levels of the corresponding group. Mothers of preterm had significantly higher mean plasma zinc level as compared to the mothers of term infant ($P < 0.001$). Non pregnant level of plasma zinc (120±7.7 ug/dl) were higher as compared to maternal zinc level in all the three groups. There was statistically no significant difference in the mean plasma zinc level

level of full term SGA infants from the value obtained for the term AGA infants.

Worker opined that decrease zinc binding capacity in maternal blood and increased transference of free zinc from the mother to the fetus leads to increased zinc level in fetus and decreased level in mother. Mean plasma zinc level in premature infants were lower than in full term AGA and SGA infants because of transference of zinc in the last trimester of pregnancy, the fact, which explain that the level of plasma zinc in fetus would increases with increasing maturity and would consequently be lower in infants born prematurely. Further relatively less alteration in zinc binding occurs at premature gestation due to lesser increase in sex hormone, which resulted in higher level of total plasma zinc and a less rise of free zinc than at term.

Dorothy Mc Master et al (1983), did an longitudinal study of the first year of life for serum copper and zinc levels in the preterm infants. Serial determination were carried out on 27 male and 21 female preterm infant (gestational age 28-36 weeks) through out the first year of life. Most of the infants were fed evaporated milk for at least 12 weeks. Solid food was introduced by 18 weeks and by 6 months. Serum copper and zinc was estimated at birth, at 9 week, 24 week and at 52 week. Estimation of copper and zinc was done by

atomic absorption spectrophotometry.

Author observed that the mean level of serum copper and zinc at birth were 0.33±0.20 ug/ml and 1.03±0.16 ug/ml respectively. Serum copper level showed an steeprise for first 9 weeks from a value of 0.33±0.20 ug/ml at birth to a value of 0.77±0.19 ug/ml, suggesting that either there was good retention of copper from the cow's milk based formulae or a redistribution of the body copper were taking. For the next 9 weeks solid was introduced and there was little change in serum copper perhaps because the intake of copper from mixed diet were just sufficient to balance the demand. After that, level continued to rise and reached 1.2±0.27 ug/ml at the end of 52 weeks. Adult levels of serum copper were attained before the child were 6 month old. Serum zinc at birth was 1.03±0.16 ug/ml; at 9 weeks, 0.71±0.11 ug/ml; at 24 weeks, 1.02±0.20 ug/ml and at 52 weeks, 1.19±0.34 ug/ml These figures showe that zinc level fell from first 9 weeks and then recovery occurred steadily and level reached to that found at birth by the age of 24 weeks. Over the remainder period serum zinc continued to rise to a final value of 1.19±0.34 ug/ml.

Author inferred that an estimation of serum zinc in preterm and full term infants at 6 month of age might be usefull as a mean of detecting zinc deficiency resulting from malabsorption or an inadequate dietary supply.

Gupta et al (1984), recently studied the serum copper, zinc, magnesium and calcium level in cord blood of north Indian neonates. They included in the study, 92 neonates of known gestation born of spontaneous vaginal delivery. They were further grouped as term AGA, term SGA, preterm AGA and post term AGA according to criteria laid down by Singh et al (1974). The cord blood was collected from cut end of the umbilical cord. Estimation of trace elements were done by atomic absorption spectrophotometry.

Author observed that the level of zinc, copper, magnesium and calcium were 212.5±3.53 ug/dl, 82.0±2.82 ug/dl, 31.7±0.70 mg/dl and 9.65±0.21 mg/dl respectively in postterm. Levels of zinc and copper in post term neonates were significantly higher than their levels in full term AGA (zinc 164.53±21.0 ug/dl; copper 59.01±19.13 ug/dl). Concentration of copper (47.01±12.28 ug/dl) and zinc (89.35±15.74 ug/dl) in preterm AGA were significantly lower when compared to full term AGA ($P < 0.05$). Serum level of zinc in full term SGA (116.33±5.49 ug/dl) were also lower than full term AGA ($P < 0.05$) while level of copper in full term SGA (58.6±23.08) did not show any significant difference with its level in full term AGA. Worker categorized the neonates in four groups according to their birth weight and tried to find out the relation of elements, if any, with the birth weight. The concentration of zinc and copper in infants with birth weight of <2000 gm was 92.13±25.48 ug/dl and 45.5±7.15

ug/dl respectively which were not different from their values in 2001-2500 gm birth weight (zinc 108.61±25.69 ug/dl; copper 53.07±20.72 ug/dl). The concentration of zinc and copper in infants with birth weight 2501-3000 gm (zinc 165.30±18.53 ug/dl; copper 57.83±19.66 ug/dl) and 3001-3500 gm (zinc 169.30±28.67 ug/dl; copper 64.30± 18.36 ug/dl) were significantly higher as compared to infants with ⩽2500 gm (P ⩽0.05) so, the mean concentration of allthese elements in cord blood showed a rise with increasing birth weight.

Worker observed that the serum calcium levels were unaffected by increasing birth weight and maturity of new born. The serum copper, zinc and magnesium levels had increasing trend with increasing maturity of the new born. The concentration of zinc and copper in infants with gestational age ⩽33 weeks (zinc 71.0±17.39 ug/dl; copper 42.5±5.00 ug/dl) and 34-37 weeks (zinc 94.17±12.33 ug/dl; Copper 47.98±17.03 ug/dl) were not significantly different, while serum concentration of zinc and copper in infants with gestational age between 38-40 weeks (zinc 152.16±27.24 ug/dl; copper 58.36± 21.06 ug/dl) and⩾41 weeks (zinc 173.18±28.06 ug/dl; copper 62.25±17.87 ug/dl) were significantly higher (P ⩽0.05) as compared to infant with a gestational age between 34-37 weeks.

Serum concentration of magnesium increased with the weight and gestation of the neonate but the calcium levels remained unaffected.

Author found no correlation of these elements in cord blood with the economic group, parity of mother and sex of the baby.

Yamashita et al (1985), in their recent work determined the concentration of zinc and copper and related metalloenzyme i.e. carbonic anhydrase isoenzymes (CA-I and CA-II) and Cu_2 Zn_2 superoxidedismutase (SOD) simultaneously in, maternal and cord blood at delivery. Eleven nonpregnant healthy women served as control while maternal and cord blood in 17 normal births work the cases.

The worker observed, that the total zinc concentration in maternal erythrocyte (1,228.4±30.8 ug/100 ml) was similar to controls (1,140.5±44.7 ug/100 ml) and CA-I derived zinc concentration was significantly higher ($P < 0.05$) in maternal erythrocytes (995.0±24.9 ug/100 ml). A very low total zinc concentration was noted in cord erythrocyte (247.0±10.4 ug/100 ml) and this was due to the low concentration of both CA isoenzyme. Ratio of other zinc (not CA or SOD derived) total zinc was significantly higher than that in control and maternal erythrocyte ($P < 0.05$). This indicates that about a third of zinc in cord erythrocyte was present in available form or attached to other zinc enzymes (e.g. lactate dehydrogenase and glyceral dehyde-3 phosphate dehydrogenase).

It was observed that the total concentration of copper in maternal erythrocyte was significantly lower (56.9±1.9 ug/100 ml) than in controls(65.9±3.1 ug/100 ml; P $<$0.05) but in cord erythrocytes it was significantly higher (77.2±3.9 ug/100 ml; P $<$0.05) than in control erythrocytes. This study indicated that SOD_1 concentration in human erythrocyte are constant and the variation in erythrocyte copper concentration are due to changes in other forms of copper (not SOD_1 derived).

Author also studied the plasma zinc and copper concentration in controls, maternal blood and cord blood. Mean plasma zinc concentration in maternal plasma(72.4± 4.6 ug/100 ml) was not lower than control plasma(83.4±4.6 ug/100 ml). It was statistically not significant, whereas plasma zinc level in cord blood(105.8±5.3 ug/100 ml) were significantly higher than maternal plasma (72.4±4.6 ug/100 ml). Mean plasma copper level in maternal blood was much higher (209.9±8.5 ug/100 ml) than that in control(77.7± 3.3 ug/100 ml) and cord plasma (31.5±2.7 ug/100 ml).

Author concluded that concentration of CA isoenzymes and of SOD_1 were independent of the plasma concentration of the trace elements. The concentration of these metals in maternal erythrocyte and plasma were significantly different from the respective concentration in cord erythrocyte and plasma. Simply, the concentration of these metals in maternal and cord erythrocyte contrasted with corresponding concentration in their plasma.

MATERIAL AND METHODS

MATERIAL AND METHODS

The present study was carried out in the department of Paediatrics in active collaboration with the department of Biochemistry and department of Obstetrics and Gynaecology, Maharani Laxmi Bai Medical College, Jhansi, over a period of 12 months from August, 1987 to July, 1988. Babies delivered by normal vaginal delivery in the labour room of Obstetrics and Gynaecology department were included in the study. Babies born by caesarian section were not selected for the present study.

STUDY GROUP

Our study group consisted of 67 new born babies delivered by normal vaginal delivery. Infants with congenital anomaly, and still birth were excluded from the present study.

New born babies were classified according to their gestational age and birth weight by the criteria laid down by Singh et al. (1974). On the basis of gestational age, babies were divided into three subgroups :

1. Preterm babies.
2. Term babies.
3. Post term babies.

New born babies with gestational age less than 37 weeks were included in preterm group. Term babies were of 37-41 weeks gestational age and babies with gestational age of 42 weeks and above were grouped under

post term babies group. Present study consisted of 10 preterm babies, while there were only 2 cases in the post term group.

According to birth weight, babies were further classified as follows by ascertaining their position on intrauterine growth curves.

1. Preterm (gestational age less than 37 weeks) :
   a. Appropriate for gestational age(AGA) birth weight between + 1SD and - 1 SD.
   b. Small for dates(SFD) - birth weight below 2 SD.
   c. Large for dates (LFD) - birth weight above 2 SD.
2. Term (Gestational age 37-41 weeks).
   a. Appropriate for gestational age(AGA) birth weight between + 1 SD. and - 1 SD.
   b. Small for dates (SFD)- birth weight below 2 SD.
   c. Large for dates (LFD) - birth weight above 2 SD.
3. Post term (Gestational age 42 weeks or more) :
   a. Appropriate for gestational age (AGA) birth weight between + 1 SD and - 1 SD.
   b. Small for dates (SFD) - birth weight below 2 SD.
   c. Large for dates (LFD) - birth weight above 2 SD.

The definition for the low birth weight (LBW) babies as laid down by World Health Organisation (1961) was adopted in the present study to classify the babies with birth weight i.e. 2,500 gm or less in LBW group.

OBSTETRICAL HISTORY AND PAST HISTORY

Apart from taking the history of socio-economic status, education of parents and monthly income, detailed obstetrical history was also taken into account. History regarding the parity, physically or neurologically damaged infants, abortions previous premature births, still birth, infants with major congenital anomaly, neonatal death were recorded in each case. Application of forceps at the time of previous deliveries and deliveries by LSCS were also recorded.

Great emphasis was given in each case to record the history of last menstrual period. Gestational age was calculated in complete weeks from the first day of the last menstrual period and by the physical criteria of Parr et al (1966).

ANTENATAL, NATAL AND POST NATAL HISTORY

A detailed account of history of any medical or surgical disorder viz. anaemia, convulsion, oedema, hypertension, cardiac disorder, diabetes, antepartum haemorrhage, hydramnios, exanthematous fever, syphilis, gonorrhoea were recorded. History of drug intake and addiction to narcotics smoking etc. was also taken in each case. History of immunization was also recorded in each case.

History was taken regarding the mode of delivery duration of labour, meconium staining of liquor and leaking.

PHYSICAL EXAMINATION OF NEW BORN

Apgar scoring of child was done at 1 minute and 5 minutes to detect any evidence of birth anoxia. After the birth of child, colour, heart rate, respiration, response to nasal catheterization, cry activity and tone was recorded on predesigned proforma to assess the apgar score.

Thorough clinical examination was done in each case. Skin was examined for colour, texture, elasticity, lanugo, oedema, dryness, pigmentation peeling or cracking of skin and planter creases. Babies were also examined for nipple formation, breast size, ear form, ear firmness and genitalia. Head of the new born babies were examined in detail for the size of fontanelle, overriding of skull bones, molding, presence of caputsuccedenum, craniotabes and cephalhaematoma, shape of the head and any mark of injury over head. Eyes were examined for any evidence of conjunctivitis or cataract. Detailed examination was done to find out any congenital abnormality. A thorough systemic examination of cardiovascular system respiratory system, nervous system and abdomen was also done in each case.

Anthropometric measurements viz. head circumference, length, chest circumference were recorded in the proforma. Birth weight of the new born baby was recorded with precision in each case within one hour of delivery.

Neonatal reflexes viz. feeding reflexes (Rooting, sucking and swallowing); extensor reflexes like Moros,

Tonic neck reflex, crossed extensor reflex, Galant's reflex and Perez reflex; progression reflexes like placing and stepping were examined in each case and recorded on the predesigned proforma to correlate them with gestational age of the baby. Assessment of gestational age was done by using the external characteristics of baby at birth according to the criteria laid down by Farr et al (1966). Eleven external characteristics were scored from 0-4 on the predesigned proforma and conversion of score into gestational age was done by the following formula or by the conversion table (Farr et al, 1966).

Estimated period of gestation (Wks) = $1.1201T - 0.0170T^2 + 22.937$ ('T' represents total score).

COLLECTION OF SAMPLE

Blood (20-25 ml) sample was collected from the cut end of umbilical cord from the placental side in the metal free glasstubes with due precaution to avoid contamination and haemolysis. All the glasswares used in the study were thoroughly sterilized and washed with distilled water.

Blood samples were allowed to clot at room temperature. After 60 minutes, the collected samples were centrifuged at 3000 rpm. for 30 minutes. After centrifugation, 6 ml of clear serum at the top of sample was transferred into another mineral free dried vial with due marking on it. The samples were kept in deep freezer ($-10^{\circ}C$).

METHOD FOR THE ESTIMATIONS OF ZINC AND COPPER

ZINC ESTIMATION

Serum zinc was estimated by the method of Song et al. (1976).

PRINCIPLE

Interferring trace metals are removed as insoluble iodides or hydroxides prior to complex formation of zinc with dithizone in NaOH Trichlor-acetate centrifugate. Absorbance of the chelate is read at 555 nm.

PROCEDURE

To 3 ml of serum in a metal free glass tube, 30 mg potassium iodide was added, and after mixing, 0.15ml of 100% (W/V) trichloracetic acid was added. The mixture was then shaken, allowed to stand at room temperature for 10 minutes, and centrifuged at 3.600 x g for 30 minutes so as to get about 2.5 ml clear supernatant. The pH of the supernatant was adjusted to approximately 13.5 by adding 0.1 ml 10M NaOH per ml supernatant. The mixture was allowed to stand for 15 minutes and centrifuged as above.

The precipitate, $Ca(OH)_2$, $Mg(OH)_2$, $Co(OH)_2$, $Mn(OH)_2$ and $Fe(OH)_2$ was discarded. To 0.8 ml of the supernatant 0.1 ml of 6 M HCl and 0.1 ml of saturated Tris buffer were than added in this order, followed by 0.1 ml dithizone reagent in 0.1 N NaOH. The pH of this mixture prior to addition of saturated Tris buffer should be between 1 and 8.5. Absorbance was determined at 555 nm.

The reading from a blank prepared by substituting distilled water for serum. The absorbance of test sample was estimated with the help of spectrophotometer and zinc concentration determined by standard curve, obtained with the serial dilution of zinc standard solution and drawn on a graph paper showing absorbance on 'y' axis and zinc concentration in ugm/ml on 'X' axis when dithizone was dissolved in 0.1 M NaOH solution. Finally the reading was recorded in ug/100 ml of serum.

COPPER ESTIMATION

Serum copper was estimated by the method of Ventura and King (1951).

PRINCIPLE

In serum, copper is released from its linkage to protein by means of hydrochloric acid, the proteins are precipitated by trichloracetic acid. Supernatant containing copper is treated with sodium diethyldithiocarbamate. Copper forms golden yellow coloured complex with sodium diethyldithiocarbamate which is extracted into an amyl alcohol-ether mixture and measured colorimeterically. Sodium pyrophosphate is added to prevent interference from iron.

PROCEDURE

To 3 ml of serum in a tube 1 ml of 0.1 N hydrochloric acid was added and it was warmed in boiling water. It was stirred continuously, until the mixture

began to cloud, it was then cooled. 1.5 ml of 6N hydrochloric acid was added and made to stand for 10 minutes. To the above mixture, 3 ml of 20% trichloracetic acid was added. It was mixed properly and after few minutes it was centrifuged. Supernatant fluid was removed and precipitate was washed with 3 ml of 5% trichloracetic acid. It was centrifuged again and the supernatant fluids were combined. To this supernatant fluid, 1 ml of 6% sodium pyrophosphate and 2 ml of ammonia was added, along with 1 ml of 0.4% sodium diethyldithiocarbamate. It was shaken with 5 ml of the amylalcohol-ether mixture for about two minutes to extract the copper. Amyl alcohol layer was removed and dried. by shaking it with a little powdered anhydrous sodium sulphate. It was used for test and reading of the sample was taken at 440 millimicrons. Five ml of standard was treated in the same way as the serum, standard and unknown were read against a complete blank. Calculation of serum copper concentration was done by the formula :

$$\text{Micrograms of copper per 100 ml serum} = \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times \frac{5}{3} \times 100$$

$$= \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 167.$$

OBSERVATIONS

OBSERVATIONS

A study to determine the serum levels of copper and zinc in cord blood of new born babies and their relationship to sex, gestational age and birth weight was carried out in 67 new born babies at M.L.B. Medical College, Jhansi from August 1987 to July, 1988 over a period of 12 months. Various clinical features were noted, birth weight was recorded and gestational age was assessed in the number of weeks from the first day of last menstrual period till birth of child&by the criteria laid down by Farr et al(1966), based on physical characteristics of new born baby.

A total of 67 cases were included in the present study to serve as case material. New born babies were classified according to maturity of child and there were 55 cases (82.08%) in full term group, 10 cases (14.92%) in preterm group and only 2 cases (0.08%) were in the post term group (Table 1).

Table 1

Showing case material of the present study.

Groups	No.of cases
1. Full Term	55(82.08)
2. Pre term	10(14.92)
3. Post term	2(0.08)
Total	67

(Figures in brackets are percentage).

Table 2 shows the classification of new born babies in to two groups which was done according to their birth weight. Normal birth weight group (having birth weight ≥2,500 gm) included 38 cases and low birth weight group (having birth weight <2,500 gm) included 29 cases (Table 2).

TABLE 2

Showing classification of babies into low birth weight (< 2,500 gm) and normal birth weight group (≥ 2,500 gm)

Groups	No. of cases
1. Normal birth weight group	38
2. Low birth weight group (LBW)	29
Total	67

Babies were further classified into appropriate for gestation (AGA), small for gestation (SGA) and large for gestation (LGA), according to criteria laid down by Singh et al (1974). Full term group comprised of 47 cases of AGA group and 8 cases of SGA group. However, in the preterm and post term group, all the cases were of appropriate for gestational age. It is evident from table 3 that maximum number

of cases were in full term AGA group (70.14%) and minimum number of cases were in post term AGA group (0.08%) (Table 3).

TABLE 3

Showing subgroups of study group according to gestation

Groups	No. of cases
1. Full term	
a. AGA	47(70.14)
b. SGA	8(11.94)
2. Pre term AGA	10(14.92)
3. Post term AGA	2(0.08)
Total	67

(Figures in brackets are percentage)

Distribution of cases according to gestational age in weeks is shown in table 4. Three cases were of $\leq$ 33 weeks of gestation, 11 cases were between 34-37 weeks and 6 cases were of $\geq$ 41 weeks. Maximum number of cases were in 38-40 weeks of gestation (70.14%) and minimum number of cases were below $\leq$ 33 weeks of gestation (4.47%) (Table 4).

TABLE 4

Showing distribution of cases according to maturity as assessed by gestational Age in weeks

Gestational age in weeks	No.of cases
1. ⟨ 33 weeks	3(4.47)
2. 34-37 weeks	11(16.41)
3. 38-40 weeks	47(70.14)
4. ⟩41 weeks	6(8.95)
Total	67

(Figures in brackets are percentage)

Study group was further classified sex wise into male and female groups. Accordingly there were 30 males and 37 females, having a percentage of 44.77% and 55.33% respectively (Table 5).

TABLE 5

Showing sex distribution of cases.

Sex	No.of cases
1. Male	30(44.77%)
2. Female	37(55.33)
Total	67

(Figures in brackets are percentage).

Sex distribution of cases in different groups of maturity is shown in Table 6. Preterm AGA group comprised of 5 cases each in male and female group, post term AGA group included 1 case each in male and female group, and full term SGA group included 4 cases each in both the sex group. Twenty seven female cases and 20 male cases were in full term AGA group. Except full term AGA, all other groups had equal sex distribution of cases (Table 6).

TABLE 6

Showing sex distribution in different groups of maturity.

Groups	No.of cases	Male	Female
1. Full term			
a. AGA	47	20(42.55)	27(47.55)
b. SGA	8	4(50.00)	4(50.00)
2. Preterm AGA	10	5(50.00)	5(50.00)
3. Post term AGA	2	1(50.00)	1(50.00)
Total	67	30(44.77)	37(55.33)

(Figures in brackets are percentage).

Sex distribution of cases in different groups of maturity is shown in Table 6. Preterm AGA group comprised of 5 cases each in male and female group, post term AGA group included 1 case each in male and female group, and full term SGA group included 4 cases each in both the sex group. Twenty seven female cases and 20 male cases were in full term AGA group. Except full term AGA, all other groups had equal sex distribution of cases (Table 6).

TABLE 6

Showing sex distribution in different groups of maturity.

Groups	No.of cases	Male	Female
1. Full term			
a. AGA	47	20(42.55)	27(47.55)
b. SGA	8	4(50.00)	4(50.00)
2. Preterm AGA	10	5(50.00)	5(50.00)
3. Post term AGA	2	1(50.00)	1(50.00)
Total	67	30(44.77)	37(55.33)

(Figures in brackets are percentage).

Babies were further classified according to their birth weight. Maximum number of cases (41.79%) were having birth weight between 2,501 gm to 3,000 gm. while 11 cases were between 1,500 gm - 2,000 gm, 18 cases had birth weight 2,001 gm - 2,500 gm and 10 cases were having birth weight ⩾3,001 gm(Table 7).

TABLE 7

Showing classification of cases according to their birth weight

Group	No.of cases
1. 1,500 - 2,000 gm	11(16.41)
2. 2,001 - 2,500 gm	18(26.86)
3. 2,501 - 3,000 gm	28(41.79)
4. ⩾3,001 gm	10(14.92)
Total	67

(Figures in brackets are percentage).

SERUM ZINC AND COPPER VALUES

All the results of serum zinc and copper are expressed as microgram/100 ml. It is evident from the table 8 that the mean ± S.D. values of both serum zinc and copper in normal birth weight group cases was much higher than the values of serum zinc

and copper in low birth weight group, values being statistically significant (P $<$ 0.001) (Table 8).

TABLE 8

Showing serum value of copper and zinc in babies with birth weight $<$ 2,500 gm and $\geq$ 2,500 gm with their statistical analysis

Groups (Birth weight)	No.of cases	Serum values (Mean±SD µg/100 ml, with range) Zinc	Copper
$<$ 2,500 gm(LBW)	29	64.22 - 146.10	37.22-70.33
		109.22±19.80	54.17±9.03
$\geq$ 2,500 gm(NBW)	28	141.00 - 210.32	44.00-88.20
		179.87±18.18	65.67±14.48
't'		15.16	3.752
'p'		$<$0.001	$<$0.001
d.f. = 65			

(d.f. = Degree of freedom)

Serum values in mean ± SD of zinc and copper were determined in preterm AGA, full term SGA, full term AGA and post term AGA. It was observed that Mean ± SD values of both zinc and copper showed an increasing trend in the same order. Values of copper and zinc in preterm AGA and full term SGA were lower than their values in full term AGA and post term AGA (Table 9A).

TABLE 9(A)

Showing values of serum copper and zinc in different groups of cases classified according to maturity of New born baby.

Groups	No.of cases	Serum values (Mean±SD, ug/100 ml, with range)	
		Zinc	Copper
1. Full Term			
a. AGA	47	108.00-210.32	44.24-80.20
		165.06±29.36	63.50±10.37
b. SGA	8	104.33-132.24	50.44-70.20
		119.04±9.91	59.13±7.59
2. Preterm AGA	10	64.22-108.37	38.40-65.47
		88.02±14.62	49.31±9.83
3. Post term AGA	2	204.20-208.21	85.57-88.20
		206.29±14.14	86.88±1.85

TABLE 9(B)

Showing statistical analysis of table 9(A).

Comparison between	d.f.	Serum Zinc		Serum Copper	
		't'	'p'	't'	'p'
I(a) Vs I(b)	53	4.36	<0.001	2.03	<0.05
I(a) Vs II	55	8.04	<0.001	3.96	<0.001
I(a) Vs III	47	1.96	>0.05	3.15	<0.01
I(b) Vs II	16	5.12	<0.001	2.32	<0.05
I(b) Vs III	8	8.32	<0.001	4.92	<0.01
II Vs III	10	10.47	<0.001	5.19	<0.001

(d.f. = Degree of freddom).

On statistical analysis of table 9(A) it was observed that serum level of zinc in preterm AGA was lower than fullterm AGA, SGA and post term AGA, which was statistically significant (P $<$0.001). Similarly the values of zinc in full term SGA was lower than other groups which was also statistically significant(P $<$0.001). On the contrary, full term AGA babies and post term AGA babies did not show any statistically significant difference (P $>$0.05), in serum zinc values, but it was seen that the serum values of zinc were apparently higher in post term than full term AGA, though the values were statistically insignificant (P $>$0.05).

Identical correlation and statistical significance of serum copper (like serum zinc) in different maturity groups was also observed in our study except that the difference in serum copper of full term AGA and post term AGA was statistically significant (P $<$0.01) (Table 9B).

An attempt was made to observe a correlation if any, between gestational age in weeks, to the biochemical values of serum zinc and copper (Table 10A and 10 B).

Cases were classified into 4 groups viz. I - $<$33 weeks, II - 34-37 weeks, III - 38-40 weeks and IV - $>$ 41 weeks. Highest values of zinc were observed in group IV while lowest values were seen in group I. It

is evident from the table 10(A) that the values of serum zinc were present in increasing order in group I and IV. Identical findings were also observed in serum copper values.

On statistical analysis, serum zinc values were significantly lower in group I when compared with rest of the 3 groups, similarly values of serum zinc in group II were significantly lower than the group III and IV ($P < 0.001$). No significant difference was observed statistically between serum zinc values in 30-40 weeks cases and 41 weeks cases ($P > 0.05$). On the other hand, the difference between the serum copper values of the latter two groups were statistically significant ($P < 0.01$).

Statistically significant difference was observed in serum copper values of different groups. So, there was direct correlation of serum copper and serum zinc to the gestational age of the child. Difference in serum copper values in group I and III were highly significant ($P < 0.001$).

TABLE 10(A)

Showing the values of serum copper and zinc in different groups of cases classified according to their gestational age

Gestational age (weeks)		No.of cases	Serum values (Range and Mean± S.D., ug/100 ml)	
			Zinc	Copper
I	⩽ 33	3	64.22 - 85.04	38.40-44.21
			73.19±10.70	41.00±2.95
II	34-37	11	81.80-113.50	37.22-65.47
			99.92±11.71	53.25±8.11
III	38-40	47	104.33-210.32	44.24-79.62
			161.66±30.62	63.41±9.73
IV	⩾41	6	141.00-208.21	44.00-88.20
			180.92±25.30	72.61±16.00

TABLE 10(B)

Showing statistical analysis of table 10 (A).

Comparison between			d.f.	Serum Zinc		Serum Copper	
				't'	'p'	't'	'p'
I	Vs	II	12	3.55	⩽0.01	2.50	⩽0.05
I	Vs	III	48	4.94	⩽0.001	3.94	⩽0.001
I	Vs	IV	7	6.88	⩽0.001	3.28	⩽0.05
II	Vs	III	56	6.53	⩽0.001	3.20	⩽0.01
II	Vs	IV	15	9.14	⩽0.001	3.35	⩽0.01
III	Vs	IV	51	1.47	⩾0.05	2.05	⩽0.05

(d.f. = Degree of freedom).

In present study, the cases were divided according to the sex of baby into male and female group. Serum values of copper and zinc were estimated in both the groups. It was observed that the difference between the values of zinc in male and female were statistically not significant(P $>$ 0.05). Simiarly copper values also didn't show any significant difference between the two groups (P $>$ 0.05) (Table 11).

TABLE 11

Showing serum values of copper and zinc in male and female babies with their statistical analysis

Sex of child	No.of cases	Serum values (Range and Mean ± S.D., ug/100 ml). Zinc	Copper
Male	30	64.22-208.21 148.06±40.03	37.22-88.20 61.37±13.08
Female	37	81.80-204.20 150.29±40.39	40.39-85.57 61.71±10.89
't'		0.22	0.11
'p'		$>$ 0.05	$>$ 0.05
d.f. = 65			

(d.f. = Degree of freedom)

The correlation of values of serum zinc and serum copper to the birth weight of baby was also studied. New born babies were classified into 4 groups according to their birth weight (Table 12 A). and serum values of zinc and copper were estimated in each group.

The lowest values of both zinc and copper were observed in group I (Birth weight between 1,500-2,000 gm) while highest values were observed in group IV(Birth weight $\geq$ 3,001 gm).

On statistical analysis of table 12(A), it was observed that the differences in values of serum zinc in group I and II was statistically insignificant ($P > 0.05$), while the differences in values of zinc in group I and III and IV were highly significant ($P < 0.001$). Serum zinc values in group II(Birth weight 2,001-2,500 gm) showed highly significant difference when compared with values of serum zinc in group III(Birth weight 2,501-3,000 gm) and group IV ($\geq$ 3,001 gm) ($P < 0.001$). On the contrary, the serum zinc values in group IV ($\geq$3,001 gm) didn't show any statistically significant difference when compared with group III(2,501-3,000 gm) ($P > 0.05$). Similar findings were also observed with serum copper in various groups.

It is evident from table 12 (A) and 12 (B) that the serum values of copper and zinc had direct correlation with the birth weight of new born baby.

TABLE 12 (A)

Showing serum values of zinc and copper in different birth weight groups

	Birth weight (gm)	No.of cases	Serum values (Range and Mean ± SD, mg/100 ml) Zinc	Copper
I	1,500-2,000	11	64.22-132.24	38.40-70.20
			106.54±23.44	54.18±10.67
II	2,001-2,500	18	81.80-146.10	37.22-70.33
			110.86±17.74	57.72±10.08
III	2,501-3,000	28	141.00-210.32	44.00-88.20
			179.01±19.58	63.79±9.83
IV	7 3,001	10	164.00-206.22	49.24-78.47
			182.29±14.14	66.57±10.41

TABLE 12(B)

Showing statistical analysis of 12 (A).

Comparison between	d.f.	Serum Zinc 't'	Serum Zinc 'p'	Serum Copper 't'	Serum Copper 'p'
I Vs II	27	0.52	7 0.05	0.89	7 0.05
I Vs III	37	9.84	< 0.001	2.68	< 0.05
I Vs IV	19	8.84	< 0.001	2.68	< 0.05
II Vs III	44	11.94	< 0.001	2.03	< 0.05
II Vs IV	26	10.92	< 0.001	2.20	< 0.05
III Vs IV	36	0.48	7 0.05	0.75	7 0.05

(d.f. = Degree of freedom).

D I S C U S S I O N

DISCUSSION

The present work was carried to study the serum level of zinc and copper in cord blood of 67 newborn babies. The study was conducted at M.L.B. Medical College, Jhansi in the department of Paediatrics from August, 1987 to July, 1988. The primary aim of our study was to evaluate serum zinc and serum copper in the cord blood of preterm, term and post term babies and to observe the correlation, if any, between the levels of these elements to the gestational age, birth weight and sex of the newborn baby. Further it was our endeavour to observe the difference in the serum level of these elements, if any, between the low birth weight babies (Birth weight $\leq$2,500gm) and normal birth weight babies (Birth weight $\geq$2,500 gm).

Besides evaluating serum zinc and copper, weight was recorded and thorough physical and general examination of the newborn baby was done at the time of birth. Gestational age was calculated in each case by counting the number of weeks from the first day of last menstrual period till the birth of child and also by the criteria based on physical characteristics (Farr et al., 1966).

Statistical analysis was done to derive mean$\pm$SD values and mean values were compared using student 't' test and the significance of difference ('p' value) was noted. Based on observations depicted in various tables (1 to 12) various inferences have been drawn which are discussed here in detail.

A total of 67 cases were examined in the present study. The cases were classified in to preterm, term and post term group. We further divided our cases into appropriate for gestational age (AGA), small for gestational age (SGA) and large for gestational age (LGA) babies. Our study comprised of 10 cases of preterm AGA and 2 cases of post term AGA, while full term group comprised of 47 AGA and 8 SGA infants. We have classified these cases according to the criteria laid down by Singh et al. (1974). Gupta et al. (1984), like us, had also grouped their cases according to the criteria of Singh et al (1974). Sann et al (1980), had classified their cases into small for gestational age when their birth weight was below the 10th percentile of Lubchenco's growth chart. However, some of the other workers (Henkin et al., 1971; Prasad et al, 1974; Dorothy Mc Master et al., 1983) have clamsified their case material according to gestational age in preterm and term infants without taking into the consideration of birth weight of newborn baby.

The cases were also classified into low birth weight group (Birth weight $\leq$2,500 gm) and normal birth weight group (Birth weight $\geq$2,500 gm) according to the definition of low birth weight given by WHO in 1962. Like us, Bagden et al (1978), Atinmo et al. (1980) and Gupta et al (1984) classified the cases into LBW and normal birth weight by adopting the same criteria laid down by WHO.

It is evident from Table 3 that maximum number of cases were in full term AGA group (70.14%) while only 2 cases were in post term group (0.08%).

Cases were also classified according to the gestational age (in number of weeks) of babies. Maximum number of cases (70.14%) were between 38-40 weeks of gestation, while only 4.47% cases were of ≤ 33 weeks of gestation. We also divided the cases in to 4 groups according to the birth weight as shown in table 7. Maximum number of cases were between 2,500 - 3000 gm (41.79%). Like us, Gupta et al (1984), had also grouped the cases according to gestational age (in weeks) and birth weight to study the relationship of serum copper and zinc in cord blood to the age and birth weight of child.

Our study included 30 male and 37 female newborn babies we also tried to observe the sex distribution in different groups of maturity. Table 6 revealed that equal number of male and female cases were present in preterm AGA, full term SGA and post term AGA group, while percentage of male and female in full term AGA was 42.55% and 57.45% respectively. Prasad et al (1974), studied 44 cases out of which 22 were male and 22 were female.

SERUM COPPER AND ZINC

RELATION WITH MATURITY OF NEWBORN BABIES

Serum zinc and copper values were observed in 47 full term AGA and 8 full term SGA infants and as is evident from Table 9(A) they had mean±SD values of serum

zinc 165.06±29.36 ug/100 ml in term AGA and 119.04±9.91 ug/100 ml in term SGA infants. The value of serum copper was 63.50±10.37 ug/100 ml in term AGA and 59.13±7.59 ug/100 ml in term SGA infants. It is evident, that the values of serum copper and zinc in full term SGA infants were significantly lower (P $<$0.001) than their values in full term AGA infants. Another important finding of our study was that serum copper (49.31±9.83 ug/100ml) and serum zinc (88.02±14.62 ug/100 ml) values in preterm AGA infants were significantly lower than their values in full term AGA infants (serum zinc 165.06±29.36; and serum copper 63.50±10.37). These differences were statistically significant (P $<$0.001). Two cases in post term group showed apparently higher values of zinc (206.29±14.14 ug/100 ml) than fullterm AGA infants (165.06±29.36) but this difference was statistically insignificant(P $>$0.05) However, serum copper value in postterm AGA (86.88±1.85) was higher than full term AGA, values being statistically significant (P $<$0.01).

So, an interesting finding of our study was that the values of serum copper and serum zinc showed an increasing trend in preterm, full term and post term infant in the same order, which signified that the values of serum copper and zinc had direct correlation with the maturity of infant. Also, full term SGA infants showed significantly lower values of serum copper and zinc when compared with their values in full term AGA infants.

Statistically insignificant differences in values of serum zinc in post term AGA and full term AGA might be due to the less number of cases (only 2 cases) which formed the post term group of our study.

Neri et al (1969), reported the value of serum copper in full term infant to be 65 ug/100 ml. Henkin et al (1971), subsequently in their study observed serum zinc and serum copper in full term AGA infants to have mean ±SD value of 83±3 ug/100 ml and 29±3 ug/100 ml respectively, which were lower as compared to the values of serum zinc and copper observed in our study. Schenker et al (1972), observed more or less similar values of serum copper in full term infants (58±10.1 ug/100 ml). However, the difference in value of serum copper in premature infants (56±11.3 ug/dl) and full term infants were statistically insignificant (P >0.05) which is not compatible with our finding. Like us, Schenker et al (1972), also found higher values of serum copper in post mature infants (65±15.6 ug/dl) than full term infants. Prasad et al (1974) on the contrary, observed no significant difference in values of serum zinc in full term AGA (100.8±34.5 ug/dl) and full term SGA infants (97.5±26.5 ug/dl). Hillman (1981), like us, observed lower values of serum copper in premature infant (26±16.8 ug/100 ml) than full term infant (32±12 ug/100 ml).

Goel and Misra (1982), like us, observed that the mean value of plasma zinc obtained in the premature AGA infants (90.2±5.25 ug/100 ml) was significantly lower

than the mean plasma zinc level in full term AGA infants (111.1±9.35 ug/100 ml) and full term SGA infants (106.57 ±7.39 ug/100 ml). It has been reported in literature that the values of zinc are 16% higher in serum than in plasma (Foley et al, 1968) and the higher results obtained in our study may be primarily due to serum rather than plasma used for estimation of these elements in our study in addition to some differences in the method used.

Gupta et al (1984), like us, also found lower values of serum zinc (89.35±15.74 ug/100 ml) and serum copper (47.0±12.28 ug/100 ml) in preterm AGA infants and also in full term SGA infants (zinc 116.33±5.49 ug/100 ml; copper 58.6±23.08 ug/100 ml). In both, these groups serum level of copper and zinc were significantly lower ($P < 0.05$) than theirvalues in full term AGA (zinc 164.53 ±21.0 ug/100 ml; copper 59.01±19.13 ug/100 ml). Our observations of higher serum zinc and copper values in post term AGA group were in conformity with the values observed by Gupta et al (1984) (zinc 212.5±3.53 ug/100 ml; copper 82.0±2.82 ug/100 ml) and with the values observed by Schenker et al. (1972).

Direct correlation of serum values of zinc and copper with increasing maturity were also observed by Gupta et al (1984) and Goel et al (1982).

Further according to the birth weight of the child serum copper and zinc was also studied in low birth weight group (≤2.500 gm) and normal birth weight group (>2.500 gm). It is evident from Table 8 that both serum

copper and zinc in low birth weight group (copper 54.17 ±9.03 ug/100 ml; zinc 109.22±19.80 ug/100 ml) were significantly lower than their values in normal birth weight group (copper 65.67±14.48 ug/100 ml; zinc 179.87 ±18.18 ug/100 ml). Our observation, that both serum copper and zinc values are lower in LBW group are in conformity with the results observed gy Gupta et al(1984).

Atinmo et al (1984) like us, also observed lower values of zinc in low birth weight group (zinc 83.37± 15.0 ug/100 ml) than normal birth weight group (89.57± 13.01 ug/100 ml), which were found to be statistically significant from each other. Some other workers however, didn't find any difference in values of serum zinc and copper in LBW and normal birth weight group (Neri et al, 1969; Bogden et al, 1978).

RELATION WITH GESTATIONAL AGE

Serum values of zinc and copper were also studied in different groups divided according to their gestational age in weeks. Highest percentage of cases were between 38-40 weeks (70.14%). New born babies of ≤ 33 weeks age had much lower values of both copper (41±2.95 ug/100 ml) and zinc (73.19±10.70 ug/100 ml) than the values observed in 38-40 weeks group (copper 63.41±9.73 ug/100 ml; zinc 161.66±30.62 ug/100 ml) as well as the values of both these elements in the other two groups viz 33-37 weeks, ≥ 41 weeks.

Similarly, serum values of both these elements were lower in new born babies of gestational age between 34-37 weeks than the values observed in babies born between 38-40 weeks and $\geq$41 weeks. Serum zinc values in babies born between 38-40 weeks (161.66$\pm$30.62 ug/100 ml) didn't show any statistically significant difference ($P \geq 0.05$) from the values observed in babies of $\geq$41 weeks gestation (180.92$\pm$25.30 ug/100 ml), though the values were higher in the latter group. Similarly serum copper level in former group (63.41$\pm$9.73 ug/100 ml) was lower than the value observed in latter group (72.61$\pm$16.0 ug/100 ml), differences in the values of copper were however found to be statistically significant ($P < 0.05$).

It is evident from our study, that serum values of copper and zinc showed an increasing trend with the increase in gestational age, implying a direct correlation of serum values of these elements with the gestational age of the new born baby. Gupta et al (1984), like us, also observed a direct correlation between gestational age and serum values of zinc and copper. Except this, no other study is available till date to find such a relation between the values of these two elements and gestational age of the newborn baby. Gupta et al (1984), like us, observed lowest value of serum zinc (71.0$\pm$17.39 ug/100 ml) and serum copper (42.5 $\pm$5.0 ug/100 ml) in babies with gestational age $<$ 33 weeks. Highest values of these elements were observed in babies with gestational

age 7 41 weeks (zinc 173.18±28.06 ug/100 ml; copper 62.25±17.87 ug/100 ml).

RELATION WITH SEX

Serum zinc and serum copper were also estimated in newborn babies according to their sex, by dividing them into two groups viz. male and female. It was observed that the differences in values of serum zinc in 37 female babies (150.29±40.39 ug/100 ml) and 30 male babies (148.06 ±40.03 ug/100 ml) were statistically insignificant(P 70.05) Similarly, serum values of copper in female babies (61.71 ±10.89 ug/100 ml) and male babies (61.37±13.08 ug/100 ml) were statistically insignificant (P 70.05). Like us, no other workers in the field have found statistically significant difference in values of both these elements in cord blood of male and female children (Schenker et al 1972; Kurz et al, 1973; Gupta et al, 1984; Henkin et al, 1971; Canzler et al, 1972 and Prasad, 1974). However Prasad et al, (1974), observed that the serum level of zinc in mothers of female children (43.0±33.2 ug/100 ml) were lower than its level in mothers of male children (62.3± 15.6 ug/100 ml), which was found to be statistically significant, however, they observed that the difference in values of serum zinc in cord blood of male and female children were not significant (male 73.2±24.8 ug/100 ml; female 83.7±83.7 ug/100 ml).

Author inferred that lower level of serum zinc in mothers of female children were presumably because of

higher need of zinc by female fetus for growth. It was supported by the fact that zinc concentration was higher in amniotic fluid of mothers of female baby than in mothers of male baby.

RELATION WITH BIRTH WEIGHT

It was our endeavour to study the relation of serum copper and zinc to the birth weight of child. In accordance we divided our cases into 4 groups as shown in Table 12(A). Lowest value of copper (54.18±10.67 ug/100 ml) and zinc (106.54±23.44 ug/100 ml) were observed in babies with birth weight between 1,500-2,000 gm and highest value observed were in babies with birth weight >3,001 gm (copper 66.57±10.41 ug/100 ml; zinc 182.29± 14.14 ug/100 ml). Difference in values of serum zinc and copper in babies with birth weight between 1,500-2,000 gm and babies with birth weight between 2,001-2,500 gm was statistically not significant (P >0.05), though their values were relatively higher in latter group (copper 57.72±10.08 ug/100 ml; zinc 110.86±17.74 ug/100 ml). Similarly, values of zinc and copper in babies with birth weight >3,001 gm (zinc 182.29±14.14 ug/100 ml; copper 66.57±10.41 ug/100 ml) were apparently higher than their values in babies with birth weight between 2,501-3,000 gm. (zinc 179.01±19.58; copper 63.79±9.83 ug/100 ml) but the difference were statistically insignificant (P >0.05). However, on comparison of the serum values of copper and zinc in babies with birth weight >3,001 gm and the values

higher need of zinc by female fetus for growth. It was supported by the fact that zinc concentration was higher in amniotic fluid of mothers of female baby than in mothers of male baby.

RELATION WITH BIRTH WEIGHT

It was our endeavour to study the relation of serum copper and zinc to the birth weight of child. In accordance we divided our cases into 4 groups as shown in Table 12(A). Lowest value of copper (54.18±10.67 ug/100 ml) and zinc (106.54±23.44 ug/100 ml) were observed in babies with birth weight between 1,500-2,000 gm and highest value observed were in babies with birth weight 3,001 gm (copper 66.57±10.41 ug/100 ml; zinc 182.29± 14.14 ug/100 ml). Difference in values of serum zinc and copper in babies with birth weight between 1,500-2,000 gm and babies with birth weight between 2,001-2,500 gm was statistically not significant (P 0.05), though their values were relatively higher in latter group (copper 57.72±10.08 ug/100 ml; zinc 110.86±17.74 ug/100 ml). Similarly, values of zinc and copper in babies with birth weight 3,001 gm (zinc 182.29±14.14 ug/100 ml; copper 66.57±10.41 ug/100 ml) were apparently higher than their values in babies with birth weight between 2,501-3,000 gm. (zinc 179.01±19.58; copper 63.79±9.83 ug/100 ml) but the difference were statistically insignificant (P 0.05). However, on comparison of the serum values of copper and zinc in babies with birth weight 3,001 gm and the values

of these elements in babies of the rest of the groups, the difference were statistically significant.

Important finding of our study was that the serum values of both the trace elements in cord blood showed an increasing trend with the increase in birth weight, suggesting an direct correlation of the values of these trace elements with birth weight of infants.

Gupta et al., (1984), like us, also observed the direct correlation of serum values of copper and zinc to the birth weight. They also observed lowest values of serum zinc (92.13±25.48 ug/100 ml) and serum copper (45.5±7.15 ug/100 ml) in babies with birth weight between 1,500 - 2,000 gm and highest values were found in babies with birth weight between 3,001-3,500 gm.(serum zinc 169.30±28.67 ug/100 ml; serum copper 64.30±18.36 ug/100 ml). These findings were more or less similar to the observations made by us.

The explanation that have been put forward to interpret the relationship of serum copper and zinc to the gestational age and birth weight of the newborn baby is discussed as follows :

In our study mean plasma zinc and copper levels in premature AGA infants were significantly lower than their values in full term AGA and post term AGA infants. Since, it is the free from of zinc and free from of copper in the maternal blood which is transported across the placenta by passive transfer (Henkin et al, 1971) and since these elements are largely being transferred during the last trimester of pregnancy (Goel et al, 1982;

Widdowson, 1969), levels of these elements in the cord blood would increase with increasing maturity and would subsequently be lower in infants born prematurely and higher in infants born at term and post term gestation. This fact is substantiated by our study in which we observed an direct correlation of serum copper and zinc level to the increasing gestational age.

It has been proved by several workers that fetus is highly dependent on the maternal serum copper and zinc level(Atinmo et al, 1980; Schenker et al, 1969; Bogden et al., 1978; Goel et al., 1982). So, any factor affecting their level in maternal blood will also affect their level in cord blood. It has been pointed out by several workers that zinc deficiency decreases the growth of not only adult male or female, but also affects the birth weight of newborn, if mothers are deficient in this metal(Atinmo et al., 1980). Our finding of significant correlation between birth weight and trace metal concentration suggests that these elements may have a possible role in occurrence of low birth weight among Indian babies. This would have very serious implications in our environment where the diets are mostly cereal based with very little contribution from animal source.

So, further work is needed to find out the contribution of dietary intake of these trace metals to the plasma status during pregnancy and in new born baby and to find out the relation with birth weight.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Present work was carried out to study the serum level of zinc and copper in new born babies. The work was conducted from August, 1987 to July, 1988, in department of Paediatrics, M.L.B. Medical College, Jhansi (U.P.). The study group comprised of 67 new born babies. The case material was divided into preterm, fullterm and post term group according to the maturity of new born babies. Further classification of babies were done into appropriate for gestation (AGA), small for gestation (SGA) and large for gestation (LGA), according to the criteria laid down by Singh et al. (1974).

The primary aim of our study was to study the serum level of zinc and copper in new born babies and to observe their correlation, if any, to the gestational age, birth weight and sex of the new born babies. It was our endeavour to compare the serum values of these elements in low birth weight group (birth weight $\leq$ 2,500 gm) and normal birth weight group ($\geq$ 2,500 gm).

Besides evaluating serum zinc and copper, therough physical and general examination was done in each case. Gestational age of the babies were calculated by counting the number of weeks from the first day of last menstrual period till the birth of baby and also by the criteria laid down by Farr et al (1966), based on physical characteristics of new born baby. Anthropometric measurements. Viz. head circumferences, chest circumference, crown to heel length and weight was recorded in each case.

SERUM ZINC AND COPPER IN NEW BORN BABIES

RELATION WITH MATURITY

Serum zinc and copper were estimated in preterm appropriate for gestational age (AGA), Full term appropriate for gestational age (AGA), Full term small for gestational age (SGA) and post term appropriate for gestational age (AGA) group. Our observations revealed that serum level of both copper and zinc were lower in preterm AGA group (zinc 88.02±14.62 ug/100 ml; copper 49.31±9.83 ug/100 ml) than their values observed in full term AGA (zinc 165.06±29.36; copper 63.50±10.37 ug/100 ml), full term SGA (zinc 119.04±9.91; copper 59.13±7.59 ug/100 ml) and post term AGA group (zinc 206±14.14 ug/100 ml; copper 86.88±1.85 ug/100 ml). Full term SGA group had lower value of serum copper and zinc than their values in full term AGA and post term AGA group.

A significant conclusion of our study was that both serum copper and zinc values showed an increasing trend with increasing maturity, which signifies that serum values of these elements had direct correlation with the maturity of new born babies.

On statistical analysis, differences in values of serum zinc in full term AGA and post term AGA were insignificant ($P > 0.05$).

It was our endeavour to observe the serum values of copper and zinc in different groups divided according to the gestational age in weeks viz. ≤33 weeks, 34-37 weeks, 38-40 weeks, ≥ 41 weeks.

It was concluded that serum values of both these elements had an direct correlation with the gestational age as their values showed an increasing trend with increasing gestational age.

RELATION WITH BIRTH WEIGHT

We divided our cases into low birth weight group (LBW) (birth weight ≤2,500 gm) and normal birth weight group (≥2,500 gm). A significant observation in our study was that serum values of copper and zinc were significantly lower in LBW group (zinc 109.22±19.8 ug/100 ml; copper 54.17±9.03 ug/100 ml) than their values observed in normal birth weight group (zinc 179.87±18.18 ug/100 ml; copper 65.67±14.48 ug/100 ml).

We also tried to observe the values of these elements in different groups divided according to birth weight viz. 1500-2000 gm, 2001-2,500 gm, 2,501-3000 gm and ≥3,001 gm.

It was concluded that serum values of copper and zinc showed an increasing trend with increase in birth weight, implying direct correlation of the values of these elements to the birth weight. The values of both these elements being lowest, in 1,500-2,000 gm group and highest in ≥ 3,001 gm group.

RELATION WITH SEX

Our study group comprised of 30 male and 37 female babies. On statistical analysis of the values of

serum zinc and copper in male babies (zinc 148.06±40.03 ug/100 ml; copper 61.37±13.08 ug/100 ml) and female babies (zinc 150.29±40.39 ug/100 ml; copper 61.71±10.89 ug/100 ml), it was found that the differences in serum values of these elements in male and female children were insignificant (P 70.05).

We are of the opinion like others for the explanation that have been put forward to interpret the relationship of serum copper and zinc values with gestational age and birth weight of the new born babies.

Our observation that serum values of copper and zinc were lower in preterm AGA infants than full term and post term infants and a direct correlation of their values with gestational age, is because of larger amount of free form of copper and zinc which is transferred passively across the placenta during the last trimester of pregnancy.

Since, the fetus is highly dependent on the maternal serum copper and zinc level and also since it has been proved that zinc deficiency decreases the growth of fetus, it is probably the nutritional status of the mother which affects the birth weight of new born. However, this finding needs to be substantiated by further studies.

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A P P E N D I X

CASE SHEET

DEPARTMENT OF PAEDIATRICS, M.L.B. MEDICAL COLLEGE, JHANSI

Case No. : M.R.D. No. :

Name : Sex : D.O.B.:

Father's Name : Time of Birth :

Address :

Mother's Name : Mother's Age :

Occupation: Father: Mother:

Total income of family: Rs. /month

Per capita income : Rs. /month

OBSTETRICAL HISTORY

Parity : Abortions :

Previous premature Births: Still Births :

Major congenital anomaly : Neonatal death :

Date of First day of last Menstrual Period :

ANTENATAL, NATAL AND POST NATAL HISTORY

Anaemia: Renal Disease:

Nutrition: Diabetes :

Convulsion: Anti partum Haemorrhage:

Oedema : Hydramnios :

Hypertension : Exanthematous Fever :

Tuberculosis : Syphilis :

Cardiac Disease :

Gonorrhoea :

H/o Drug Intake: Addiction to Narcotics, smoking etc.

H/o Leaking :

Mode of delivery : Vaginal

Caesarian

Duration of Labour :

Meconium staining of Liquor :

Spontaneous or Induced :

CLINICAL EXAMINATION OF NEW BORN BABY

APGAR SCORING

Clinical Feature	0	1	2
Colour	Blue/Pale	Peripheral cyanosis	All over pink
Respiratory efforts.	Absent	Slow irregular	Good crying
Heart rate	Absent	<100/min.	>100/min.
Activity	Limp	Some flexion of limbs	Active movements
Response to the catheter in nostril	No response	Grimace	Cough or sneez

Score: At 1 minute :
At 5 minute :

Anthropometric Measurements:

Birth weight : LBW
Head circumferences:
Chest circumference:
Crown to heel length:

Maturity Assessment

External Sign	Score 0	1	2	3	4
Edema	of hands & feet with pitting over tibia	Only pitting over tibia	No		
Skin texture	Very thin	Thin & smooth	Superficial peeling	Slight Thick superficial peeling	Thick parchment like.

Skin colour	Dark red	Uniformly pink	variable pink	pale	
Skin opacity (Trunk)	veins seen clearly	veins & tributaries seen	Few large vessels	not clear	No vessel seen
Lanugo (over back)	No	Abundant & long thick	Thin	Occasionally	More than half of back is devoid
Plantar creases	No	Faint red over anterior half	definite red over more than anterior half.	Indentation over more than ant. 3rd.	deep definite over more than ant. 3rd.
Nipple	No areola barely visible	Areola smooth ⩽0.75cm	Areola Stippled flat ⩽0.75cm	Areola stippled Edge raised ⩾0.75 cm.	
Breast size	No.	⩽0.5 cm	0.5-1 cm	⩾ 1 cm	
Ear	Pinna flat shapeless	Incurving of part of edge.	Incurving of whole edge.	Well defined incurving	
Ear Firmness	Soft, no recoil	Slow recoil	ready recoil	Firm	
Genitalia Male	Neither Testis in scrotum.	At least one testis	Both		
Female	Labia Majora widely separate	L. Majora almost covering L. minora	L.Majora completely cover L. minora.		

Total Score :

Gestational age :

Preterm : Appropriate for gestational age (AGA)

Small for gestational age (SGA)

Large for gestational age (LGA)

Full Term : AGA

SGA

LGA

Post Term AGA

SGA

LGA

General Appearance

Posture

Cry

Activity

Skin : Colour Icterous Cyanosis Pallor

Pigmentation

Nails

Mongolion spots

Peeling of skin/cracking of skin

Texture

Lanugo

Oedema

Subcutaneous fat.

Head

Fontanelle Cephal haematoma

Molding Craniotabes

Caput succedenum Shape

Injury mark

Face and Neck

Any Congenital anomaly

Neonatal reflexes

Feeding reflexes :	Rooting :
	Sucking :
	Swallowing:
Extensor reflexes :	Moros
	Tonic neck reflex
	crossed extensor reflex
	Galant's reflex
	Perez reflex
Progression reflexes	Placing
	Stepping

Cardiovascular system

Respiratory system

Central Nervous system

Abdomen:

Date	Serum Zinc	Serum Copper